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Comparative study on gill morphology of gastropods from Moreton Bay, Queensland

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Abstract

This paper reports the gill morphology of seven common gastropods from Moreton Bay, southeastern Queensland, to test the level of difference in gill structure between major taxa. The investigated species include representatives of the clades Patellogastropoda, Neritimorpha and Vetigastropda as well as representatives of the more 'advanced' Caenogastropoda. Examination by SEM and LM revealed that the external gill structure of the investigated caenogastropods (including *Planaxis sulcatus*, *Littoraria articulata*, *Bembicium auratum* and *Morula marginalba*) shows basic uniformity. The gill filaments are composed of a clearly defined ridge and an extended sheet of non-ciliated cells. The gill filaments of these species differ in the shape of the filaments (corrugated, triangular or rounded) and the presence of secretory cells. The gills of the limpet *Patelloida mimula* and neritid *Nerita chameleon*, representatives of the clades Patellogastropoda and Neritimorpha respectively, are both triangularly shaped, but differ by the presence of paddle shaped cilia in the former species and secretory cells in the latter. The gill morphology of the vetigastropod trochid *Austrocochlea constricta*, characterized by blade shaped filaments covered with nodules and a striped pattern of ciliated cells, showed the least similarities with the other investigated species.

Introduction

Gills are the principal organs of respiratory gas exchange in molluscs, and additionally perform a trophic function in most bivalves and some gastropods. Where primary gills (ctenidia) have been lost, secondary gills have evolved in some molluscan groups from modifications of the mantle surface (Yonge, 1952). Although the histological structure of gastropod gills has been well described (Hyman, 1967; Voltzow, 1994), the number of studies on gastropod gill morphology is very limited. A few papers have been published on the ultrastructure of ctenidia of bivalves (Nakao, 1975; Porvaznik *et al.*, 1979; Fisher and Hand, 1984; Giere, 1985 and Le Pennec *et al.*, 1988), while Schipp *et al.* (1979) have dealt with the ctenidia of the cephalopod *Sepia officialis*. Russell-Hunter (1988) described the functional morphology of the ctenidia of chitons, but did not detail the ultrastructure of the gill filaments. Recently, however, Fischer *et al.* (1990) have presented a detailed SEM/TEM account of the gills of *Chiton olivaceus*. The few species studied in these papers have little in common as they belong to widely separated molluscan classes. The only ultrastructural studies on gastropod gills dealt with the secondary gills of the opisthobranchs *Phyllidia pulitzeri*, *Archidoris pseudoargus* and *Peltodoris atromaculata* (Wägele, 1984; Jonas, 1986), the pulmonate *Siphonaria capensis* (De Villiers and Hodgson, 1987) and the patellogastropod *Patella vulgata* (Nuwayhid *et al.*, 1978). These species belong to different gastropod clades and, surprisingly, literature on 'prosobranch' gill morphology is lacking.

Table 1. Interspecific relationships between the investigated species taken into account the revised classification as reviewed by Haszprunar (1988) and Bieler (1992).

CLASS	SUBCLASS	ORDER	SUPERFAMILY	FAMILY	SUBFAMILY	GENUS	SPECIES
Gastropoda	Prosobranchia	Patellogastropoda	Patelloidea	Acmaeidae		*Patelloida*	*P. mimula*
		Neritimorpha	Neritoidea	Neritidae		*Nerita*	*N. chameleon*
		Vetigastropoda	Trochoidea	Trochidae		*Austrocochlea*	*A. constricta*
		Caenogastropoda	Littorinoidea	Littorinidae	Littorininae	*Littorina*	*L. articulata*
					Lacuninae	*Bembicium*	*B. auratum*
			Cerithioidea	Planaxidae		*Planaxis*	*P. sulcatus*
			Muricoidea	Muricidae		*Morula*	*M. marginalba*

In the present study the ctenidia of a number of common gastropods from Moreton Bay were investigated with SEM and LM. The purpose of selecting a range of gastropods was to test, for the first time, the level of difference in gill ultrastructure between major taxa [*e.g.* clades such as Patellogastropoda (*Patelloida mimula*), Neritimorpha (*Nerita chameleon*) and Vetigastropoda (*Austrocochlea constricta*) versus the more 'advanced' Caenogastropoda, including representatives of the families Muricidae (*Morula marginalba*), Planaxidae (*Planaxis sulcatus*) and Littorinidae. By examining two local species of the caenogastropod family Littorinidae (a world-wide and well defined group), results could be compared at the intergeneric level (*Littoraria articulata* versus *Bembicium auratum*) and also viewed in relation to the various niches occupied by littorinids within the study area. The results will be discussed in the context of the systematics of the species involved (Table 1), taking into account the latest reorganisation of the 'prosobranch' classification as reviewed by Haszprunar (1988) and Bieler (1992).

Materials and methods

Animals: All investigated species were collected during low tide at Wellington Point on the western shore of Moreton Bay, South-East Queensland, where they inhabit the intertidal zone on rocky shores and jetty piles (Eertman and Hailstone, 1988). The animals were transferred to the laboratory, where they were held in 10 l aquariums filled with seawater taken from the collection site. The water was continuously aerated and maintained at a constant temperature of 22 °C. The animals were allowed to acclimatize to laboratory conditions for at least 48 hours before the gills were removed.

Scanning Electron Microscopy: The shell of a species was carefully cracked with a vice, and then the animal removed. The gills were excised and rinsed in freshly filtered seawater, before being left in 16 % glycerol for 1 hour to remove the bulk of mucous deposits (Mariscal *et al.*, 1978). The gills were then sonicated (Unosonics sonicator) for one or two periods of 30 seconds in freshly prepared 16 % glycerol to remove any remaining mucous. After a further rinse in fresh seawater, the gills were fixed for 2 hours in 3 % glutaraldehyde, prepared with 0.1 M phosphate buffer (osmotically adjusted with 5 % sucrose, to prevent tissue damage caused by osmotic shock). As mucous particles sometimes remained attached to the surface of the gill filaments, the gills were sonicated for an additional period of 30 seconds in 5 % (v/v) NH_4OH after fixation. After a final rinse in 0.1 M phosphate buffer, the tissue was dehydrated in a graded series of ethanols and critical point dried after two changes of amyl acetate. The gills were gold-coated in a SPI sputter coater and examined using a Philips SEM 505.

Transmission Electron Microscopy: The gills were fixed for 2 hours in 3 % glutaraldehyde, prepared with 0.1 M phosphate buffer (with 5 % sucrose added). After a rinse in phosphate buffer, they were postfixed for 80 minutes in phosphate buffered 1 % osmium tetroxide. Subsequently the gills were

rinsed again in phosphate buffer, dehydrated in a graded series of ethanols, and embedded in Spurr's epoxy medium (Spurr, 1969). Ultrathin sections were cut on a LKB UM III, and stained with lead citrate and uranyl acetate (30 s Pb – 60 s UA – 30 s Pb) (Daddow, 1983). The sections were examined with a Hitachi H300 transmission electron microscope at 75 kV.

Light Microscopy: The excised gills were rinsed in freshly filtered seawater, before being fixed overnight in 10 % neutral buffered formalin. The next day the gills were washed in distilled water, then dehydrated in a graded series of ethanols (70 %, 90 % and 100 %, for periods of 30 minutes each). This was followed by two thirty minute periods of immersion in toluene, after which the gills were embedded in wax. Subsequently, 6 µm thick sections were cut on a Spencer Rotary 828 microtome. After completion of the cytochemical tests the sections were examined and photographed under an Olympus light microscope fitted with a photo-automat.

Cytochemistry: Schiff's periodic acid test was performed on 6 µm thick light microscopic sections to determine the presence of mucopolysaccharides in secretory cells (Lillie, 1964). Ayoub-Shklar's (1968) method for keratin and prekeratin was performed on 6 µm thick light microscopic sections to establish the type of connective tissue present in the gill filaments. Mayer's haematoxylin – eosin alcoholic stain was used when preparing routine overview sections.

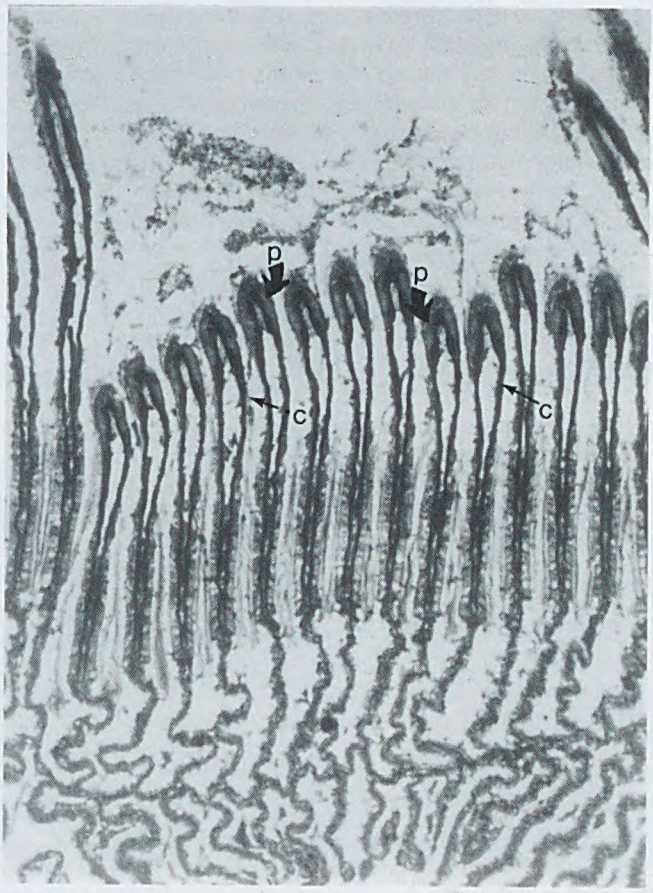

Figure 1. LM micrograph of the gill of *Austrocochlea constricta*. The nature of the connective tissue was determined with Ayoub-Shklar's (1968) method for keratin and prekeratin. The largest proportion of the connective tissue consists of collagen (c), which stains darkest. The somewhat lighter stained areas surrounding the collagen consist of prekeratin (p).

Results

Austrocochlea constricta (Vetigastropoda, Trochidae)

External gill structure: The trochid *A. constricta* has a single bipectinate gill, positioned on the left side of the mantle cavity. The anterior third of the gill is supported by a collagenous rod, which attaches the gill to the mantle wall. The filaments of the outer row are shorter (1.1 ± 0.1 mm) than those of the inner row (3.2 ± 0.1 mm). Towards the anterior and posterior ends of the gill, the filaments gradually decrease in length (Plate 1A). Each gill filament is blade-shaped, and its dorsal margin covered with long cilia (Plate 1B + 1C). A row of nodules occurs on both sides of individual gill filaments, each nodule being covered with long cilia, with the exception of the basal margin (Plate 1B, 1C + 1E). Alternating rows of ciliated and non-ciliated cells give each filament a striped appearance. The rows of ciliated cells, which are usually 3 to 4 cell-layers wide, extend from the base of a nodule across the filament towards the outer margin (Plate 1C). These cilia, which are shorter than those covering the nodules, appear in small tufts (Plate 1D).

Internal gill structure: Transverse sections through gill filaments reveal a single layered epithelium, with columnar epithelial cells that are 13.8 ± 0.7 µm long (20 cells measured). Each filament is supported axially by a double layer of collagenous connective tissue, enclosing the haemocoelic space (Plate 2A). This double layer of connective tissue thickens towards the anterior end of the filament where both layers join, forming the main haemolymph canal. The connective tissue layer surrounding the main haemolymph canal consists of an inner layer of collagen and an outer layer of proteinaceous prekeratin (Figure 1). Ciliated cells are noticeably more electron-dense than non-ciliated cells (Plate 2B). Each cilium is composed of a standard 9+2 axoneme, a basal body and associated ciliary rootlets (Plate 2B). All ciliated and non-ciliated cells exhibit microvilli on their apical surface. Although individual microvilli were not discernible under SEM, their presence is clearly demonstrated in TEM sections (Plates 1F and 2B). Microvilli measure 1.1 ± 0.1 µm in length (20 cells measured), and are surrounded by an electron-dense glycocalyx (Plate 1F). All epithelial cells, whether ciliated or non-ciliated, have a round to oval nucleus in the centre or basal half of the cell. Mitochondria seem to be concentrated in the apical half of each cell. Gap junctions between cells, containing septate desmosomes, are associated with membranes of adjoining epithelial cells and presumably serve as attachment zones (Plate 1F).

Gill filament nodules are triangularly shaped in longitudinal sections and broadly attached to the filament (Plate 2C). Each nodule has a maximum width of about 40 µm and is approximately 33 µm high. Each nodule has a coating of columnar epithelial cells of which the apical ones are ciliated. The function of these nodules remains unclear. Although some sensory function could perhaps be expected, no obvious sensory cells were observed inside the nodule.

Two types of secretory cells could be distinguished in the gills of *A. constricta*: mucous secreting cells and secretory cells containing large electron-dense vesicles. The mucous secreting cells have a typical goblet cell appearance and are located amidst the epithelial cells (Plate 2D). The cells are oval with a narrow apical neck facing towards the surface of the gill filament. The cells, which react PAS-positively, are filled with mucus in one large vacuole. The mucus appears as finely granulated secretions of low electron density. The nucleus and cell organelles are confined to a narrow area near the lateral and basal areas of the plasma membrane. The second type of secretory cell is not restricted to a specific location in the gill. It can be found: (1) bordering the surface of the filament, (2) totally enclosed by epithelial cells (Plate 2E), or (3) inside the nodules that cover the gill filaments. This type of secretory cell shows many characteristics associated with cells that have high metabolic activity. Mitochondria with well developed cristae are present throughout the cell. A rough endoplasmic reticulum (RER) occupies most of the cytoplasm (Plate 2F), indicating a high level of protein synthesis. Secretory vesicles, budded from the Golgi cisternae, presumably fuse to form the larger vesicles. All secretory vesicles, irrespective of size, are clearly surrounded by a membrane. The function and structure of these secretions remain unknown, although the cells react negatively to PAS tests, indicating that mucopolysaccharides are not present. In cells that border the gill surface, release of secretory products by exocytosis could be observed.

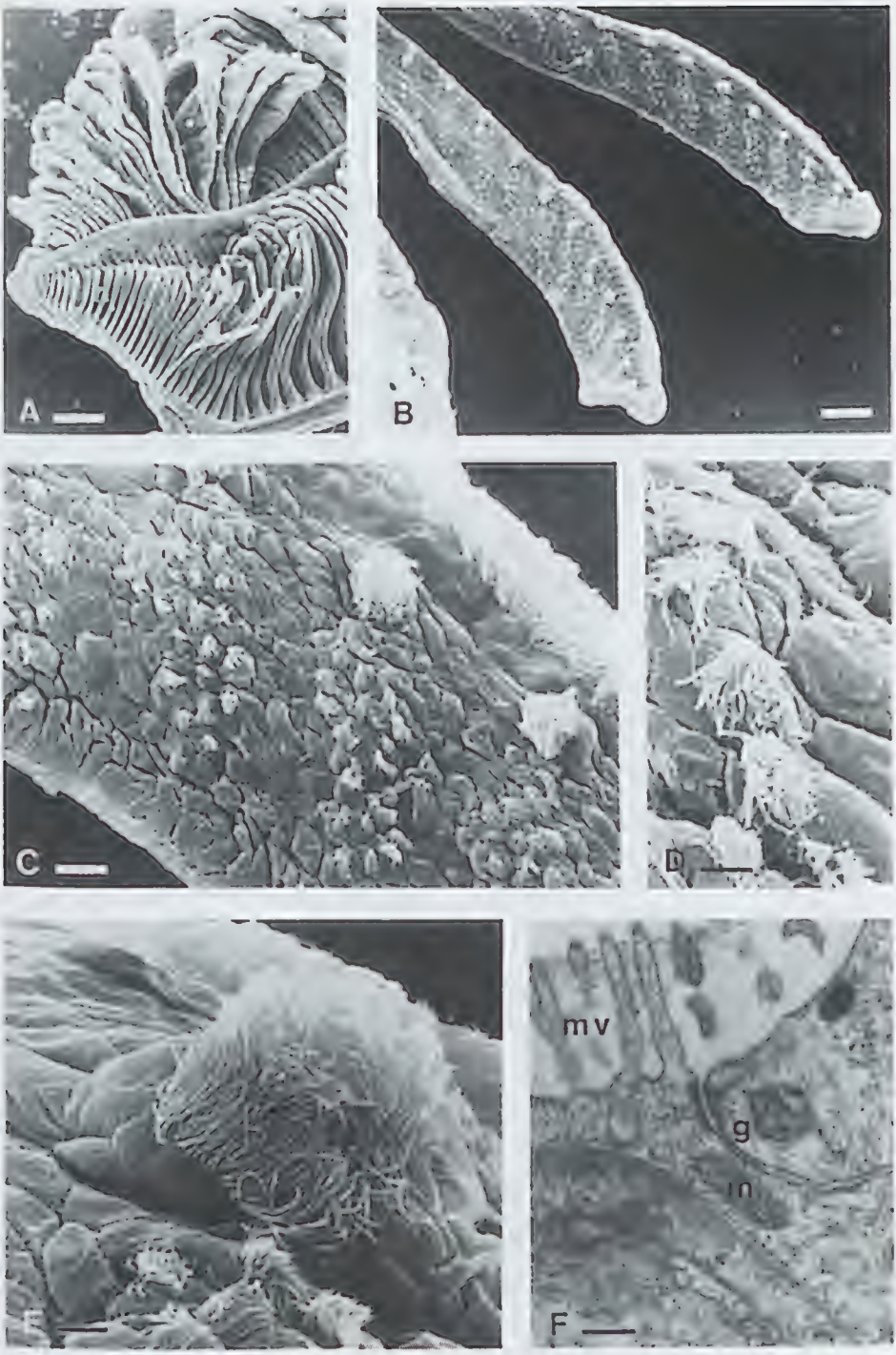

Plate 1. SEM and TEM micrographs of the gill *of Austrocochlea constricta.* A. General view showing anterior region of bipectinate gill. Bar = 0.16 mm. B. Individual gill filaments, showing rows of nodules and striped pattern of ciliated cells. Bar = 0.12 mm. C. Detail of gill filament. Bar = 15 µm. D. Detail of area with ciliated and non-ciliated cells. Bar = 4 µm. E. Nodule. Bar = 3.5 µm. F. Detail of non-ciliated cell with microvilli (mv), mitochondria (m) and gap-junction (g). Bar = 0.2 µm.

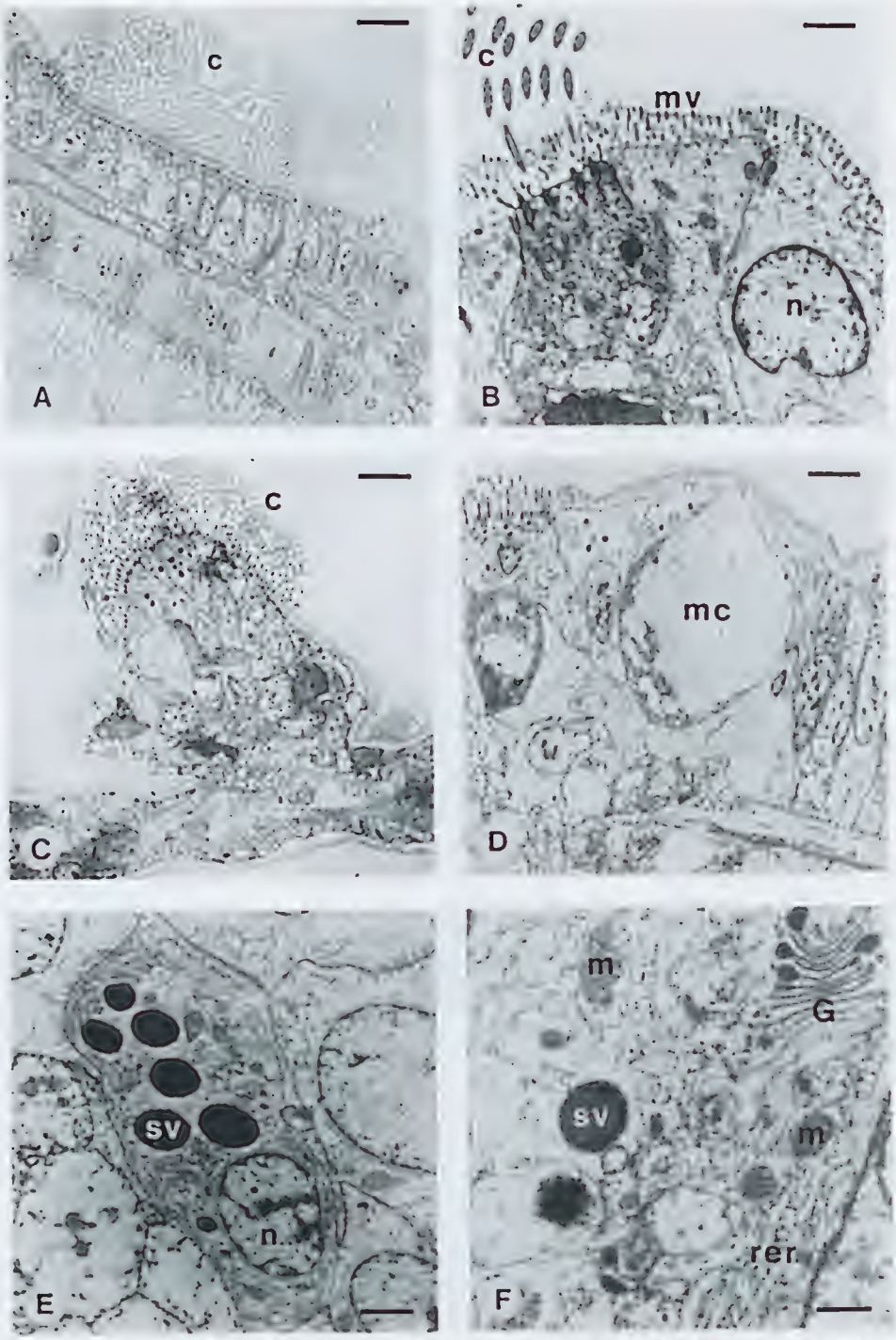

Plate 2. TEM micrographs of the gill of *Austrocochlea constricta*. A. Part of gill filament showing single layered epithelium. Bar = 10 µm. B. Ciliated and non-ciliated cells. Bar = 1 µm. C. Nodule. Bar = 7 µm. D. Mucous cell. Bar = 1.1 µm. E. Secretory cell. Bar = 1.2 µm. F. Detail of secretory cell. Bar = 0.4 µm. Key: b, basal bodies; c, cilia; G, Golgi apparatus; m, mitochondria; mc, mucous cell; mv, microvilli; n, nucleus; rer, rough endoplasmatic reticulum; sv, secretory vesicle.

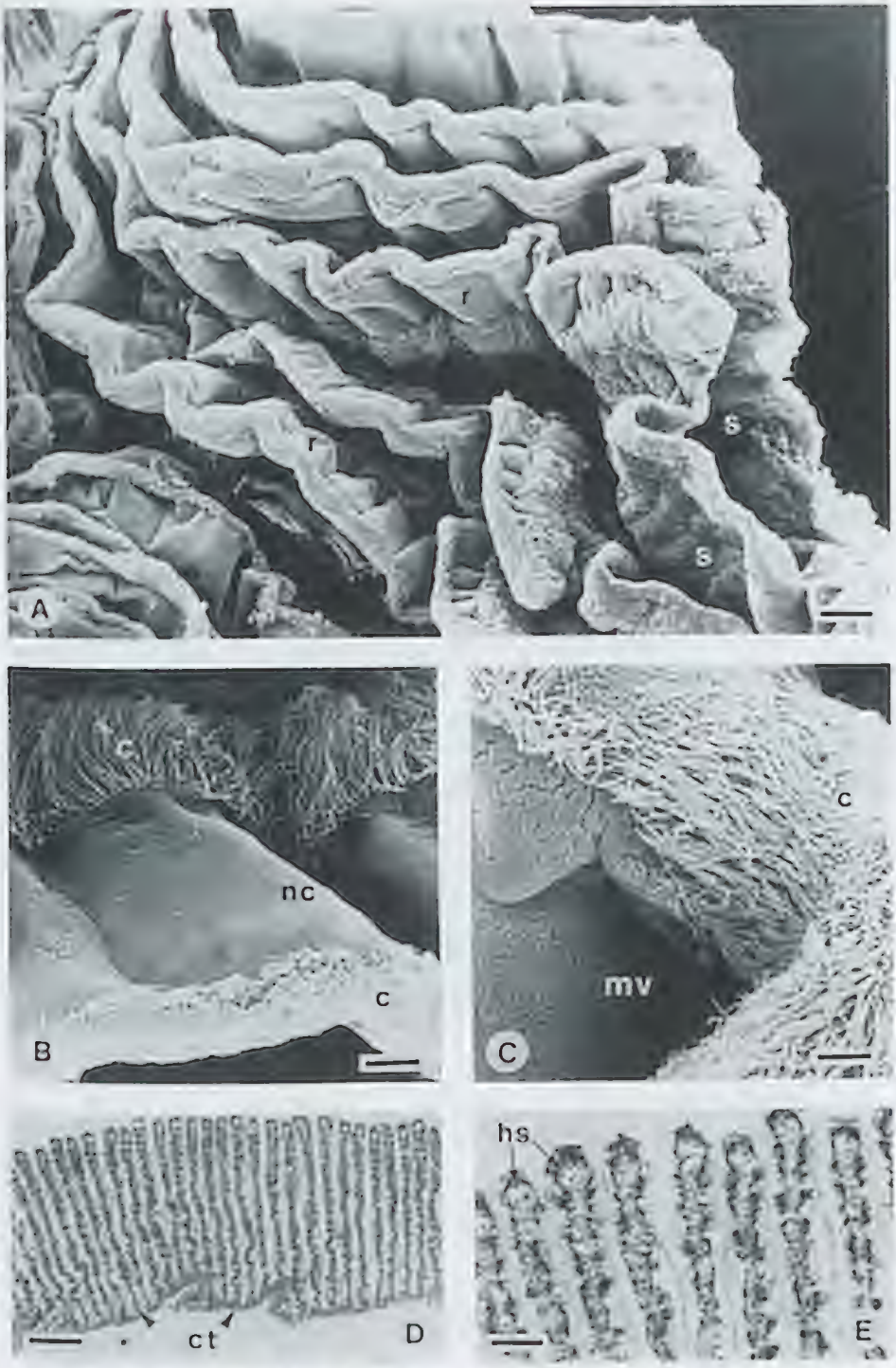

Plate 3 SEM and LM micrographs of the gill of *Planaxis sulcatus*. A. General dorsal view of gill filaments. Bar = 40 μm. B. Dorsal ridge of a filament. Bar = 13 μm. C. Detail of dorsal ridge. Bar = 4 μm. D. General view of the gill in LM section. Bar = 0.2 mm. E. Longitudinal section of filaments with haemocoelic spaces (hs). Bar = 60 μm. Key: r, dorsal ridge; s, thin sheet; c, cilia; nc, non-ciliated cells; mv, microvilli; ct, connective tissue.

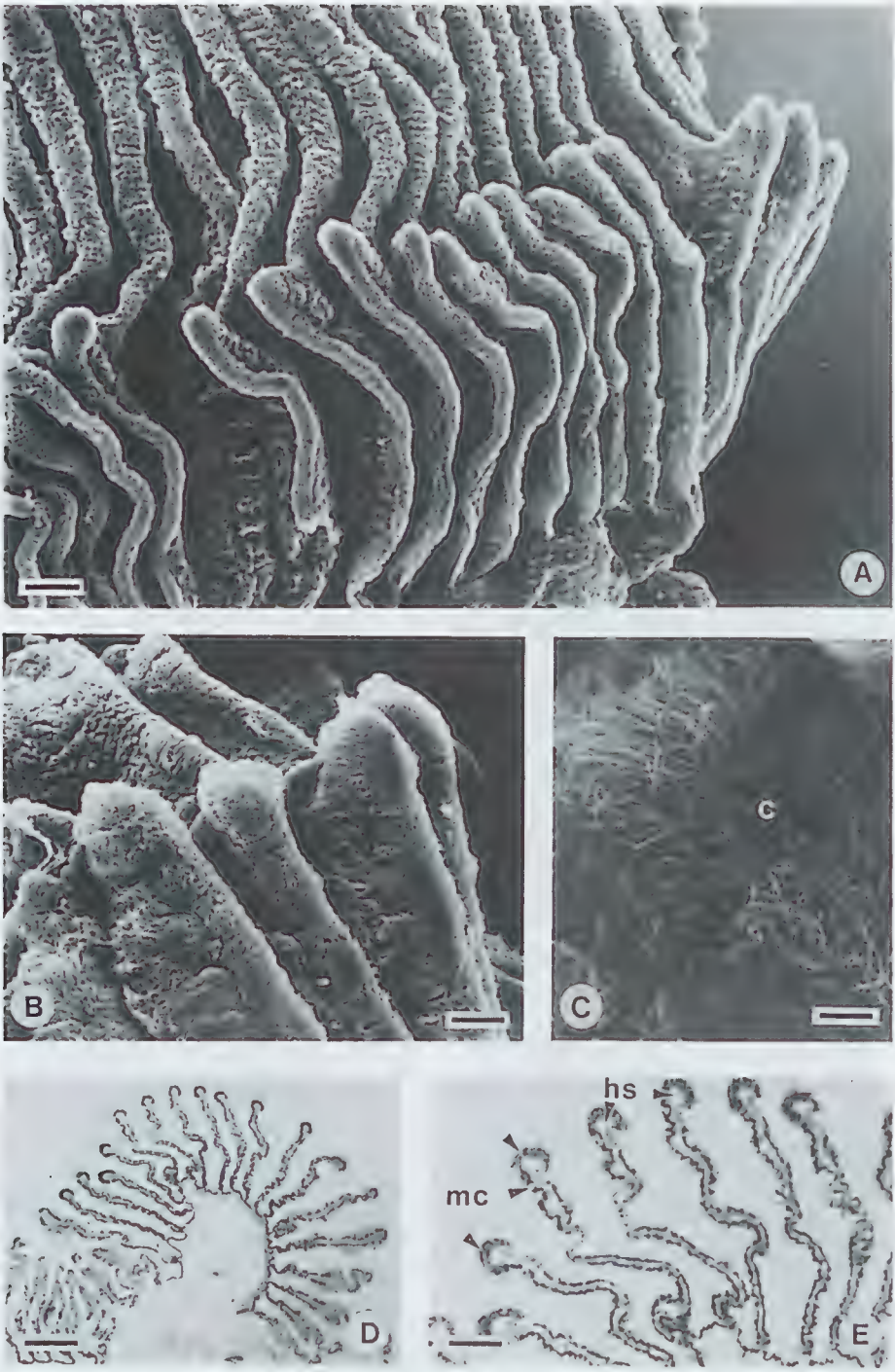

Plate 4. SEM and LM micrographs of the gill of *Littoraria articulata*. A. General dorsal view of the gill. Bar = 70 µm. B. Detail of a few filaments. Bar = 30 µm. C. Detail of one filament with border area between ciliated and non-ciliated cells. Bar = 2 µm. D. General view of the gill in LM section. Bar = 0.2 mm. E. Longitudinal section of filaments. Bar = 75 µm. Key: r, latero-dorsal ridge; s, thin sheet; c, cilia; mc, mucous cell; hs, haemocoelic space.

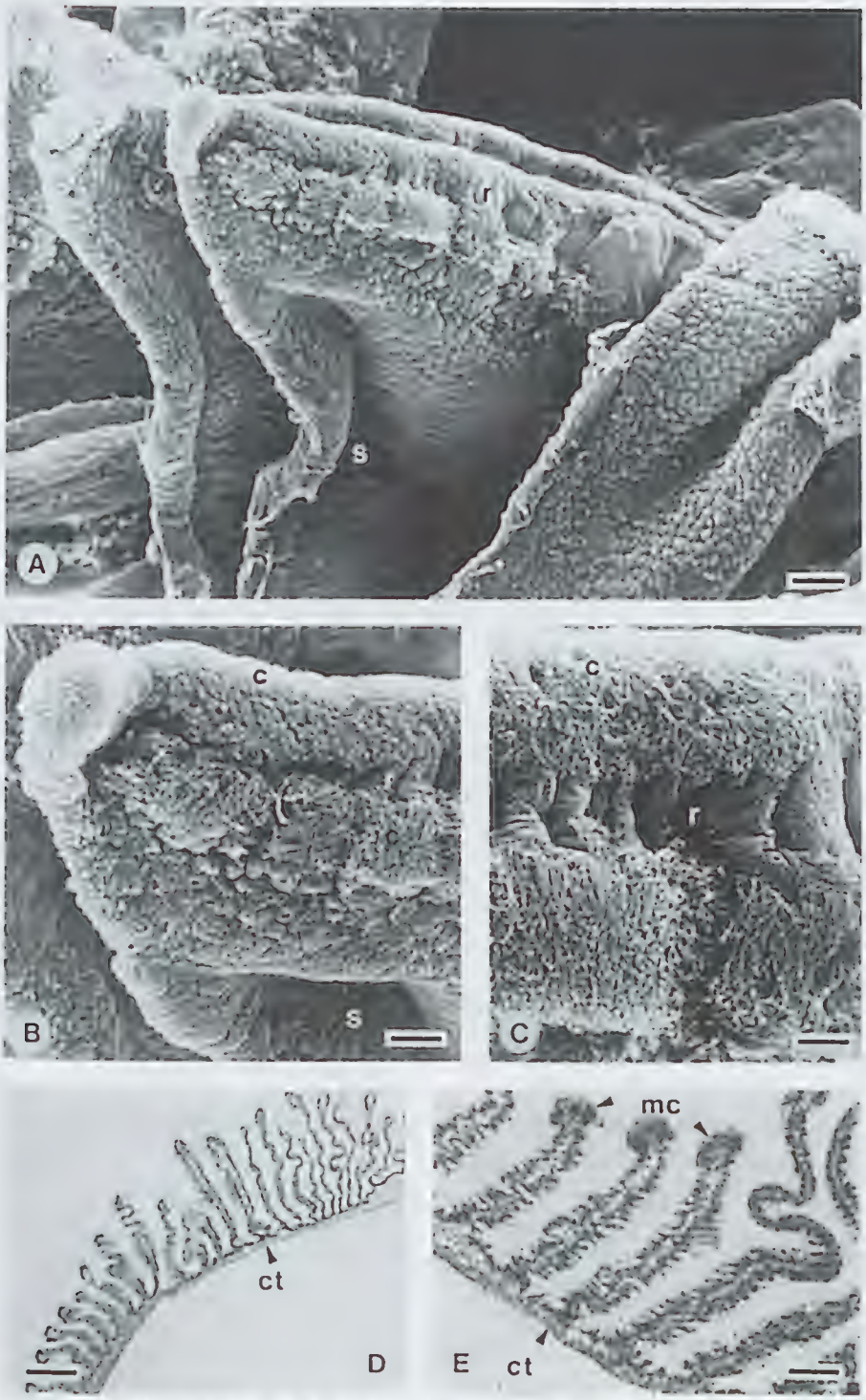

Plate 5 SEM and LM micrographs of the gill of *Bembicium auratum*. A. General lateral view of gill filaments. Bar = 40 µm. B. Detail of dorsal region of a gill filament. Bar = 20 µm. C. Detail of dorsal ridge. Bar = 13 µm. D. General view of the gill in LM section. Bar = 0.2 mm. E. Longitudinal section of gill filaments. Bar = 55 µm. Key: r, dorsal ridge; s, thin sheet, c, cilia; ct, connective tissue; mc, mucous cells.

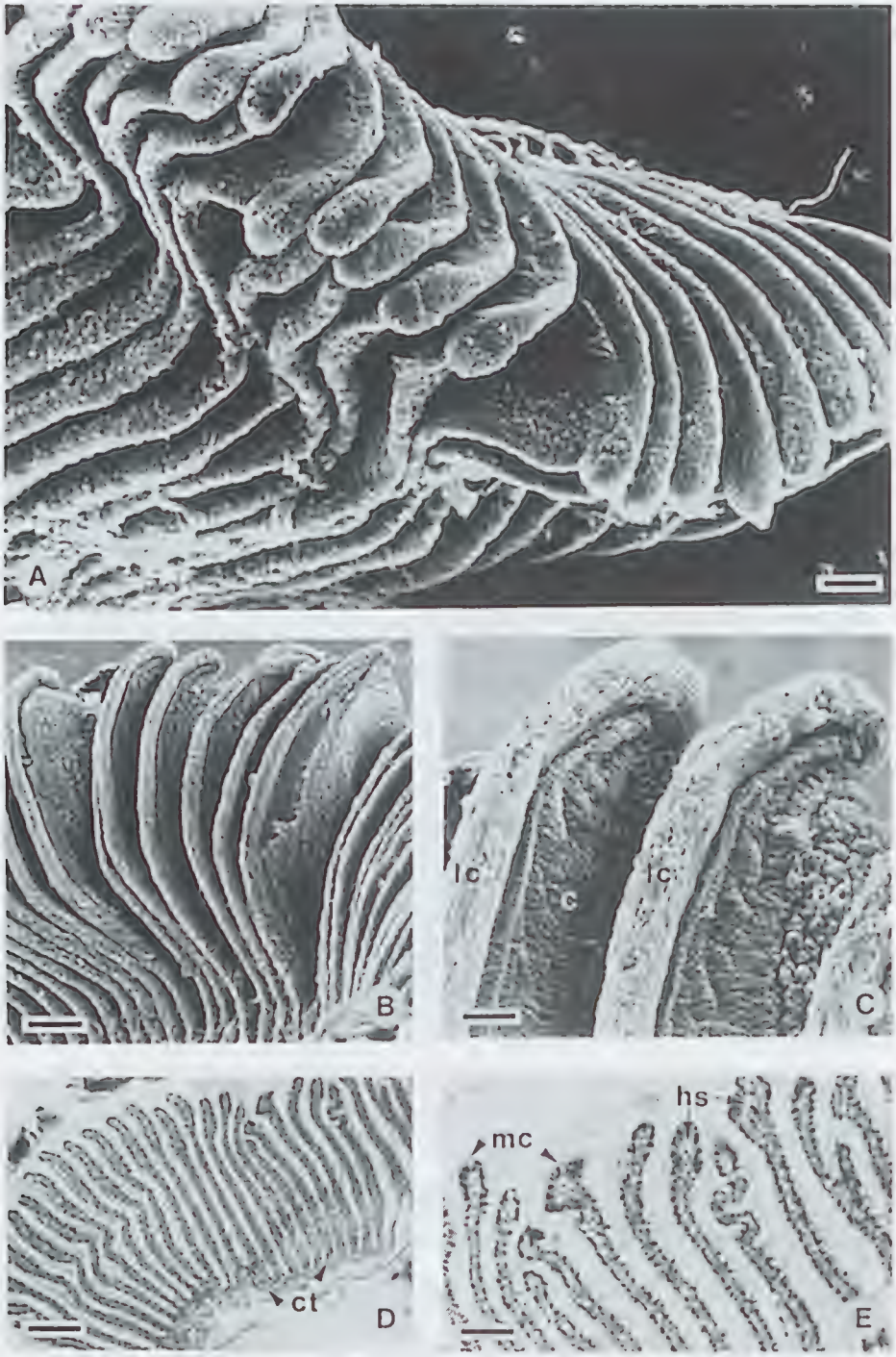

Plate 6. SEM and LM micrographs of the gill of *Mytilus marginalba*. A. General dorsal view of gill. Bar = 55 µm. B. Lateral view of a number of filaments. Bar = 0.14 mm. C. Detail of two filaments. Bar = 35 µm. D. General view of gill in LM section. Bar = 0.2 mm. E. Longitudinal section of gill filaments. Bar = 80 µm. Key: lc, long cilia; c, cilia; nc, non-ciliated cells; ct, connective tissue; mc, mucous cells; hs, haemocoelic space.

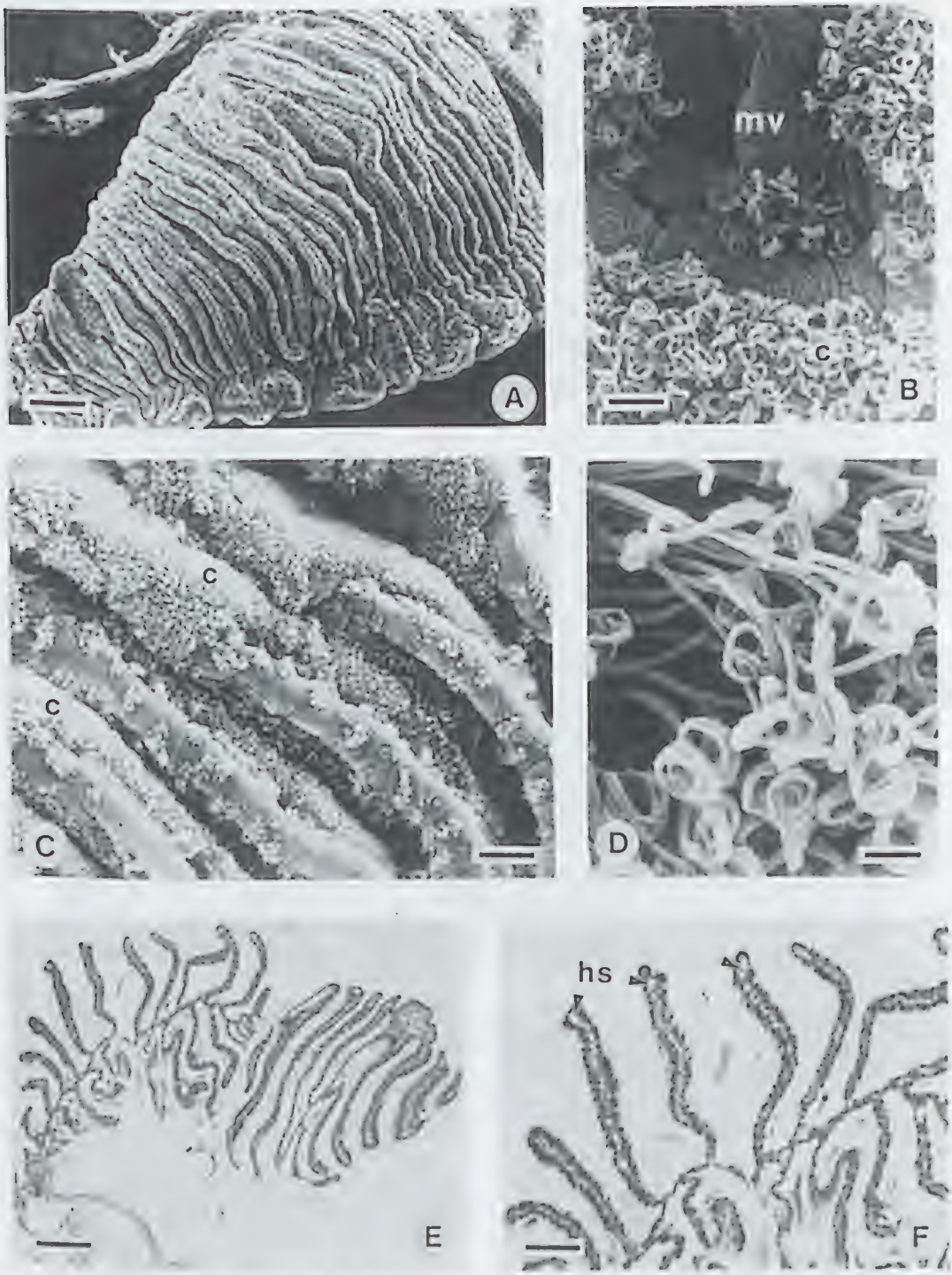

Plate 7 SEM and LM micrographs of the gill of *Patelloida mumula*. A. General dorsal view of the gill. Bar = 0.15 mm. B. Part of filament with ciliated and non-ciliated cells. Bar = 4 µm. C. Detail of a number of filaments. Bar = 25 µm. D. Paddle shaped cilia. Bar = 1 µm. E. General view of gill in LM section. Bar = 0.2 mm. F. Longitudinal section of gill filaments. Bar = 95 µm. Key: mv, microvilli; c, paddle shaped cilia; hs, haemocoelic space.

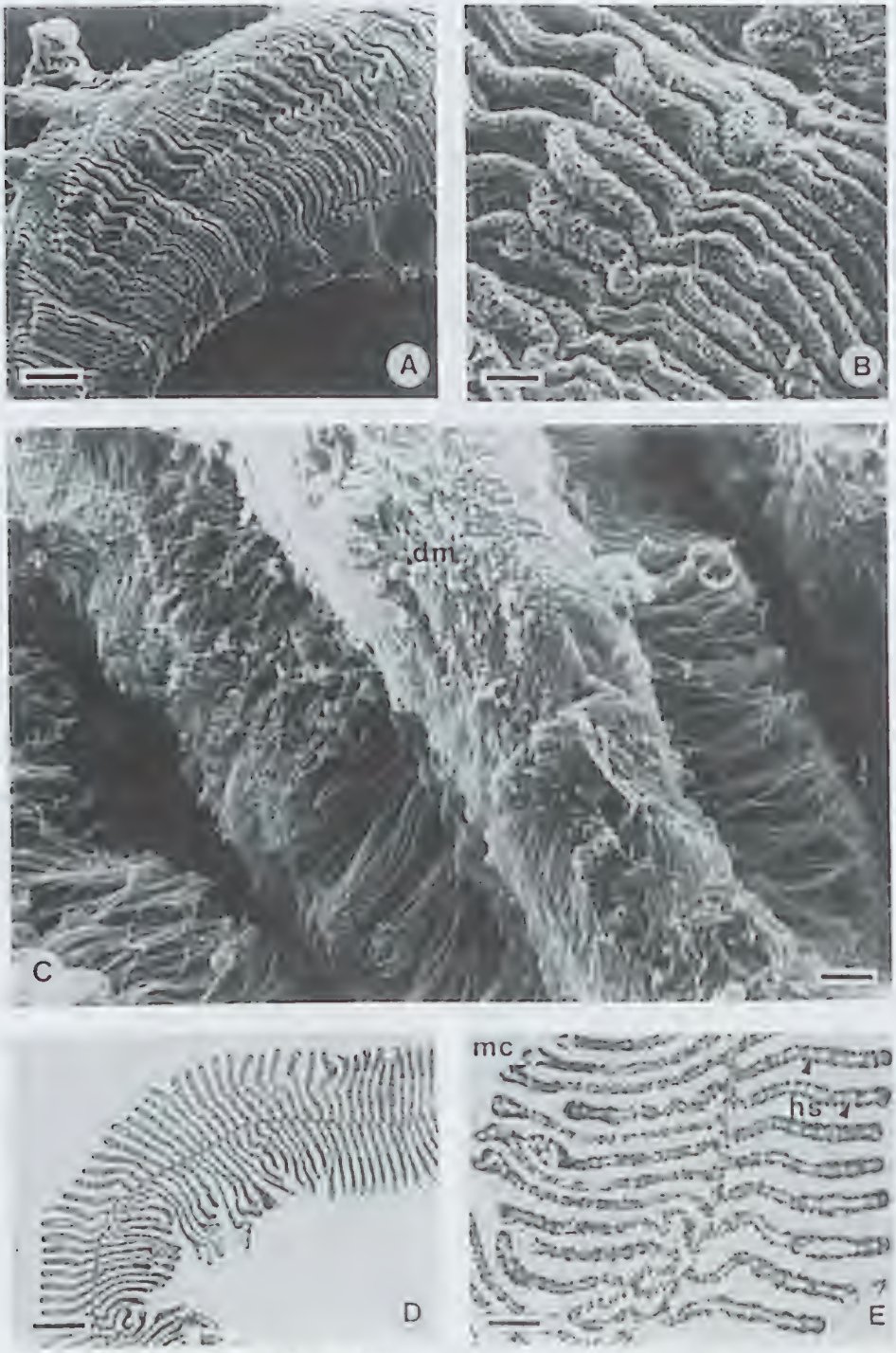

Plate 8. SEM and LM micrographs of the gill of *Nereis chamaeleon*. A. General dorsal view of the gill. Bar = 0.15 mm. B. Detail of a number of filaments. Bar = 55 μm. C. Detail of one filament. Bar = 0.5 mm. D. General view of gill in LM section. Bar = 0.15 mm. E. Longitudinal section of gill filaments. Bar = 55 μm. Key: dm, dorsal margin; mc, mucous cell; hs, haemocoelic space.

Planaxis sulcatus (Caenogastropoda, Planaxidae)

The caenogastropod *P. sulcatus* has a single monopectinate gill, positioned on the left side of the mantle cavity, attached to the mantle wall. The gill filaments extend from the anterior margin of the mantle deep into the mantle cavity. They are positioned parallel to each other and appear morphologically identical, although the size of the filaments gradually decreases posteriorly. Each filament has a narrow dorsal ridge, which extends ventrally as a thin sheet of tissue (Plate 3A). Each ridge is corrugated and covered at the margins with long cilia (Plate 3B). The central area of the ridge, consisting of non-ciliated cells, has a very smooth appearance and its surface is covered with microvilli (Plate 3C). The thin sheet of tissue extending from the dorsal ridge consists of non-ciliated cells and has a rougher appearance than the non-ciliated surface of the ridge (Plate 3A). Light microscopic sections confirm the parallel orientation of the gill filaments and show that the filaments are joined together at their base (Plate 3D). Haemocoelic spaces can be observed inside individual filaments (Plate 3E). Connective tissue, present inside the filaments and along the base where filaments fuse, presumably provides structural support. No secretory cells could be observed (Plate 3E).

Littoraria articulata (Caenogastropoda, Littorinidae, Littorininae)

In the caenogastropod *L. articulata*, a single monopectinate gill is present, positioned on the left side of the mantle cavity, and all gill filaments appear morphologically identical (Plate 4A). Each filament possesses a latero-dorsal ridge, which extends as a thin sheet of tissue across the mantle cavity. This gives each filament a somewhat triangular and elongated appearance (Plate 4B). The margins of the latero-dorsal ridge are covered with long cilia, while the cells of the elongated sheet are non-ciliated (Plate 4B + C). Light microscopic sections show that all filaments are linked to a common base (Plate 4D). In each filament a haemocoelic space is visible, forming a wider, round canal anteriorly inside the latero-dorsal ridge. Goblet type mucous cells are present in the anterior part of the filaments (Plate 4E).

Bembicium auratum (Caenogastropoda, Littorinidae, Lacuninae)

The single monopectinate gill of the caenogastropod *B. auratum* is attached to the mantle wall on the left side of the mantle cavity and consists of triangular shaped filaments (Plate 5A). All filaments appear uniform in shape, but from the anterior side onwards they gradually increase in size, and posteriorly a small reduction occurs. The dorsal surface of the filament is formed by a ridge showing numerous irregular folds (Plate 5C). The margins of the ridge consist of ciliated cells, surrounding a central area of non-ciliated cells, which has a rougher appearance than the corresponding area in *P. sulcatus*. The remaining part of the filament is formed by a thin sheet of tissue, composed of non-ciliated cells (Plate 5A + B). Light microscopic sections show that all filaments are joined together at the base, which is supported by connective tissue (Plate 5D). Each filament shows a haemocoelic space and anteriorly a well developed group of mucous cells (Plate 5E). It can also be clearly seen that although the filaments appear uniform in shape, they differ substantially in size.

Morula marginalba (Caenogastropoda, Muricidae)

The caenogastropod *M. marginalba* has a single monopectinate gill, positioned on the left side of the mantle cavity. The anterior part of the gill is not aligned with the mantle edge, but was observed more posteriorly in the mantle cavity. The gill filaments lie parallel to each other and are slightly curved (Plate 6A + B). The rounded dorsal ridge of each filament, extending from the base to the apex, is covered with long cilia. Adjacent to the ridge, a second area of ciliated cells is present, although these cilia are shorter than the previous ones (Plate 6C). The remaining part of the filament is formed by a sheet of tissue consisting of non-ciliated cells and has a rough appearance. Light microscopic sections reveal that the double layer of connective tissue present inside the gill filaments, not only encloses the haemocoelic space, but also extends into the base where filaments merge (Plate

6D). The widened anterior parts of the filaments, representing the rounded edges (Plate 6C), form the major haemolymph canals. Mucous cells, closely resembling goblet cells, can be observed in the anterior part of the filaments (Plate 6E).

Patelloida mimula (Patellogastropoda, Acmaeidae)

The gill of the acmaeid limpet *P. mimula* is triangularly shaped, pointing anteriorly (Plate 7A). It is positioned in the centre of the mantle cavity, extending forward to the mantle margin and attached to the mantle by a thin membrane. The individual filaments lie parallel to each other, are uniform in shape and increase in size posteriorly. Large areas of the filaments are covered by cilia, which differ from the ordinary type by the presence of terminal paddles (Plate 7B + D). The paddles appear to consist of a solid outer margin covered on one side by a thin membrane. Ciliated and non-ciliated cells are not organized in any recognizable pattern (Plate 7C). Large uninterrupted areas of ciliated cells are alternated with areas that consist largely of non-ciliated cells, but that include numerous small tufts of ciliated cells. Light microscopic sections show that the gill of *P. mimula* is not monopectinate, as it appears to be in SEM preparations, but bipectinate. All filaments join in the centre, although in an alternating rather than strictly paired fashion (Plate 7E + F). In the centre of each filament the haemocoelic space is visible. No secretory cells were observed.

Nerita chameleon (Neritimorpha, Neritidae)

The neritamorph *N. chameleon* has an elongated triangular shaped gill, positioned slightly left of centre in the mantle cavity and pointing anteriorly (Plate 8A). The gill is attached to the mantle by a thin membrane. All filaments lie parallel to each other and are corrugated (Plate 8B). The dorsal margin and lateral surfaces of each filament are covered with cilia. However, cilia associated with the lateral surfaces are longer, and orientated differently from those of the dorsal margin (Plate 8C). Light microscopic sections show that *N. chameleon* has a bipectinate gill, whose filaments are linked to a central base. The linkage is unpaired and the left and right filaments alternate. A haemocoelic space can be seen in the centre of each filament and towards the anterior end goblet type mucous cells were observed.

Discussion

External ultrastructure: The present study demonstrates pronounced differences in ctenidial morphology among the investigated gastropod species from Moreton Bay. The results show that species belonging to the clades Patellogastropoda (*P. mimula*), Neritimorpha (*N. chameleon*) and Vetigastropoda (*A. constricta*) possess bipectinate gills, whereas species belonging to the more advanced Caenogastropoda (*L. articulata*, *B. auratum*, *P. sulcatus* and *M. marginalba*) have monopectinate gills. The gills of Vetigastropoda, a group to which the trochid *A. constricta* belongs, are characterized by the presence of a skeletal rod, which has a supportive function. In *A. constricta* the gill filaments of the outer row are much shorter (1.1 ± 0.1 mm) than those of the inner row (3.2 ± 0.1 mm). This modification, which was observed also in other Trochoidea species and in the gills of the related vetigastropod family Lepetodriloidea, is considered to be a transitional condition from a bipectinate to a monopectinate gill structure (Haszprunar, 1988). Gill filaments covered with rows of nodules and regular patterns of ciliated and non-ciliated cells, as observed in *A. constricta*, have not been reported in ctenidia of other species. Randomly distributed clusters of cilia were observed on the lamellae of the secondary gills of the limpet *P. vulgata* (Nuwayhid *et al.*, 1978) and the pulmonate limpet *Siphonaria capensis* (De Villiers and Hodgson, 1987). However, from a taxonomic – phylogenetic perspective ctenidial structures cannot be compared to features of secondary gills, as these two types of gills are not homologous. Although there are three ciliated areas on the gill filaments of *A. constricta* (margin – nodules – striped pattern of ciliated cells), it is unclear as to what extent they function to generate water currents, disperse mucous and other gill secretions, or aid in cleansing of the gill filaments.

The lack of a skeletal rod in the bipectinate gills of the acmaeid limpet *P. mimula* and the neritid *N. chameleon* may be considered a primitive condition (Haszprunar, 1988). The gill structure of these representatives of the clades Patellogastropoda and Neritimorpha is remarkably similar. The gill of *P. mimula* is more distinctly triangular in shape in comparison to the gill of *N. chameleon*, but in both species the gill is positioned in the centre of the mantle cavity, the gill filaments lie parallel to each other and the linkage of the left and right filaments to the base is alternating.

The gills of the caenogastropod species *P. sulcatus*, *L. articulata*, *B. auratum* and *M. marginalba* have a basically uniform composition, although species specific variation occurs. Each gill filament consists of a clearly defined (latero-)dorsal ridge, which extends as a thin sheet of tissue across the mantle cavity. The shape of the filaments shows species specific differences. The gill filaments of the closely related littorinids *L. articulata* and *B. auratum* both appear triangular in shape, although the gill filaments of *L. articulata* are very much more elongated than those of *B. auratum*. The gill filaments of the muricid *M. marginalba* are slightly curved, giving the gills a quite distinct appearance. The gill filaments of the planaxid *P. sulcatus* are recognizable by the corrugated nature of the dorsal ridge, which may be a modification to enlarge the total gill surface area and improve respiratory gas exchange.

The gill filaments of all investigated species possessed areas of ciliated alternating with areas of non-ciliated cells. In general it can be stated that the main gill margin of all investigated species consisted of ciliated cells. In cases where the gill filaments possess a clearly defined ridge, as in the caenogastropods *P. sulcatus*, *L. articulata* and *B. auratum* and *M. marginalba*, both the dorsal and ventral margins of the ridge consist of ciliated cells. If present, the enclosed area of the ridge has a wavy appearance and is non-ciliated. The dorsal ridge of *M. marginalba* differs from this general pattern by not having a central area of non-ciliated cells. In this species the outer margin of the ridge is covered with long cilia, bordered by an area of cells that bear shorter cilia. The cilia were of the ordinary straight type with sharp terminals in all species except the acmaeid limpet *P. mimula*, where each cilium had a paddle shaped terminal. Similar cilia have been observed only in the secondary gills of the siphonariid limpet *S. capensis* (De Villiers and Hodgson, 1987), but not in the common patellid limpet *P. vulgata* (Nuwayhid *et al.*, 1978). Paddle shaped cilia have been reported to occur in molluscan osphradia, where they perform a chemoreceptive function (Haszprunar, 1985). However, De Villiers and Hodgson (1987) were unable to detect any nerve connections to these cilia in the pallial gills of *S. capensis* suggesting that their only function is as water paddles, as originally proposed by Yonge (1952). It has been demonstrated in *P. vulgata*, that movement of cilia creates a water current in the opposite direction to the haemolymph flow through the gill filaments, thus establishing a counter current mechanism for respiratory gas exchange (Nuwayhid *et al.*, 1978). The flattened tips of the cilia of *P. mimula* would be expected to magnify the strength of the water current, increasing the efficiency of the cilia as water paddles. Ciliary movement also takes part in distributing mucous secretions, that serve to capture foreign particles and remove them from the gills.

Internal structure: In terms of internal structure, gastropod gills are less disparate than their external ultrastructure suggests. Light microscopical sections showed that gill filaments of all species studied have a standard internal architecture. Whether gills were mono- or bipectinate, individual filaments showed basic similarities. All filaments were positioned parallel to each other and were linked by a common base, through which the haemolymph is directed and distributed to the individual filaments for respiratory gas exchange. Each filament consists of a unicellular epithelium, presumably to enhance a rapid gas exchange, and is supported by connective tissue which partly encloses the haemocoelic space. When comparing the ultrastructure of the gill epithelium of *A. constricta* with data from literature one should realise that most studies dealt with species that possess secondary gills. A single layered epithelium, consisting of either columnar or cuboidal cells, has been found in most species studied so far: *S. capensis* (De Villiers and Hodgson, 1987), *P. vulgata* (Nuwayhid *et al.*, 1978), the nudibranchs *Archidoris pseudoargus* and *Peltodoris atromaculata* (Jonas, 1986), the freshwater bivalve *Anodonta woodiana lauta* (Nakao, 1975). However, there appears to be a difference in the thickness of the epithelial cells,

which ranges from 4 µm for the cuboidal cells in the gill of *S. capensis* (De Villiers and Hodgson, 1987) to 10 – 50 µm for the columnar cells in the gills of the nudibranchs *A. pseudoargus* and *P. atromaculata* (Jonas, 1986). The columnar cells in the gill epithelium of *A. constricta* have an average thickness of 13.8 µm, which is similar to the thickness of the columnar cells in the bivalve *Lucina costata* (Giere, 1985). De Villiers and Hodgson (1987) suggested that there is a direct relationship between the thickness of epithelial cells and the degree of protection. Unprotected gills would be prone to desiccation and therefore would need to have thicker epithelial cells. This may be the case for some species, but the present results do not support this theory. Although the columnar cells in the gills of *A. constricta* could be classed as being moderately thick, the gill itself is located inside the mantle cavity and therefore is well protected from any desiccating influence.

Mucous secreting cells were observed in all species except *P. sulcatus* and *P. mimula*. They were usually of the ordinary goblet type except in the littorinid *B. auratum*, where the mucous cells were larger and were grouped in the anterior region of the filament. The presence of mucous cells and other secretory cells in gill filaments is not uniform throughout molluscan species. In the gills of the common limpet *P. vulgata* (Nuwayhid *et al.*, 1978) and the freshwater bivalve *A. woodiana lauta* (Nakao, 1975), mucous cells or other secretory cells were not observed. Jonas (1986), however, located three types of granular gland cells and an additional mucous cell in nudibranchs. The TEM study into the internal gill ultrastructure of *A. constricta* showed that the goblet type mucous cells contain one mucus-filled vacuole and, like the surrounding epithelial cells, possess microvilli. The structure of the mucous cells is comparable to those described previously. The second type of secretory cell observed in the gill of *A. constricta* has not been reported in any other molluscan gill. Large membrane bound secretions are present throughout the cell. Since this cell is found totally enclosed by other cells or bordering the filament surface, it is unclear whether the secretory products are secreted externally, or into the haemolymph or both. Further cytochemical study is certainly needed to clarify the functioning of this cell type. De Villiers and Hodgson (1987) suggested that the possession of mucous cells may be related to gill location. Exposed gills, as observed in the pulmonate *S. capensis*, in contrast to internal gills would not need mucous cells, as ciliary currents would be adequate to keep the lamellae clear of debris. The gills of all investigated species in this study are well protected inside the mantle cavity and the absence of mucous cells in *P. sulcatus* and *P. mimula* does not agree with Hodgson's suggestion. The presence or absence of mucous cells does not seem to be related directly to the habitat of the investigated species, as mucous cells were observed in species inhabiting the shore at low to mid tidal level (*A. constricta*, *M. marginalba*) as well as in species inhabiting the shore at high tidal level (*L. articulata*) (Eertman & Hailstone, 1988).

Concluding, it can be said that apparent species specific differences in external gill ultrastructure do not imply functional differences between gills. Each species has developed its own gill morphology presumably to optimize respiratory gas exchange. Features that characterize gill

Table 2. A summary of the features that characterize the ctenidia of the investigated gastropods.

Species	Clade	Type of gill	Uniform rows of filaments	Clearly defined dorsal ridge	Skeletal rod/ nodules	Shape of filaments	Paddle shaped cilia	Secretory cells
A. constricta	Vetigastropda	bipectinate	–	–	+	blade	–	+
P. mimula	Patellogastropoda	bipectinate	+	–	–	*	+	–
N. chameleon	Neritimorpha	bipectinate	+	–	–	*	–	+
P. sulcatus	Caenogastropoda	monopectinate		+	–	corrugated	–	–
L. articulata	Caenogastropoda	monopectinate		+	–	triangular	–	+
B. auratum	Caenogastropoda	monopectinate		+	–	triangular	–	+
M. marginalba	Caenogastropoda	monopectinate		+	–	curved	–	+

* Individual filaments do not have a characteristic shape.

morphology of the investigated species are summarized in Table 2. The external ultrastructure of the investigated caenogastropod species showed basic uniformity, as did the gills of the limpet *P. mimula* and neritid *N. chameleon*, representatives of the Patellogastropoda and Neritimorpha respectively. The gill ultrastructure of the vetigastropod trochid *A. constricta* showed the least similarities with the ultrastructure of the gills of the other species investigated.

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